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TOTAL AROMATIC AMINO ACID REQUIREMENT OF THE INDIAN MAJOR CARP, *CIRRHINUS MRIGALA* (HAMILTON-BUCHANAN)

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Key words: aromatic amino acid requirement, carp, phenylalanine, tyrosine

Abstract

Quantification of the essential amino acid requirements of a species is required for formulating optimal diets for cultivated fish. In this study, crystalline L-phenylalanine was used to determine the quantitative phenylalanine requirement of juvenile *Cirrhinus mrigala.* Test diets (40% crude protein) contained vitamin-free casein and gelatin supplemented with crystalline L-amino acids to provide an amino acid profile similar to *C. mrigala* muscle protein except for phenylalanine. Diets containing six graded levels of L-phenylalanine (0.80, 1.10,1.40, 1.70, 2.00 and 2.30%) and a fixed level of tyrosine (0.52%) were formulated and fed to triplicate groups of *C. mrigala* juveniles twice a day at a rate of 10% of the fish body weight per day for 56 days. The optimum dietary requirement for phenylalanine, estimated by breakpoint regression analysis, was 1.75% of the diet (4.38% of the dietary protein) or 2.27% of the total aromatic amino acid (5.68% of the dietary protein). Beyond the optimum level, phenylalanine significantly depressed growth. The best feed efficiency, specific growth rate and survival were obtained with the diet containing close to the optimum level of aromatic amino acid.

Introduction

Fish do not have a true protein requirement, but need a well-balanced mixture of indispensable and dispensable amino acids (Wilson and Halver, 1986). The amino acid profile of a diet dictates the biological value of the protein. Since indispensable amino acids are not synthesized by fish, their intake through diet is essential. The same ten essential amino acids required by other animals are needed by fish for maintenance and growth. The quantification of the essential amino acid (EAA) requirements of a species is needed to formulate diets that optimize protein utilization.

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Phenylalanine and tyrosine are aromatic amino acids. Adequate amounts of both are needed for proper protein synthesis and other physiological functions in fish. Fish can readily convert phenylalanine to tyrosine or use dietary tyrosine to meet the metabolic need for this amino acid. Therefore, to determine the total aromatic amino acid requirement (phenylalanine + tyrosine), the dietary requirement for phenylalanine is determined either in the absence of tyrosine or with test diets containing very low levels of tyrosine.

So far, the quantitative amino acid requirements for all ten essential amino acids have been investigated for only a few fish species namely, common carp (Nose, 1979), Japanese eel (Arai et al., 1972), channel catfish (Wilson et al., 1978), Chinook salmon (Halver et al., 1959), Nile tilapia (Santiago and Lovell, 1988), milkfish (Borlongan and Coloso, 1993), Mozambique tilapia (Jauncey et al., 1983) and catla (Ravi and Devaraj, 1991) and these studies indicate differences among species (Moon and Gatlin, 1991; Borlongan and Coloso, 1993).

Cirrhinus mrigala is a commercially important freshwater fish, widely reared in polyculture in India. The economic success of controlled production of *C. mrigala* depends largely on the cost of feeds, particularly protein sources used in the diet. Dietary requirements of *C. mrigala* are known for only a few indispensable amino acids, namely lysine (Benakappa and Varghese, 2002a), threonine (Benakappa and Varghese, 2002b) and tryptophan (Benakappa and Varghese, 2003). The purpose of the present study was to quantify the dietary phenylalanine requirement of *C. mrigala* juveniles at a dietary level of 0.52% tyrosine.

Materials and Methods

Experimental diets. Six isonitrogenous diets were formulated to contain 40% protein (dry matter basis) with six levels (0.80, 1.10, 1.40, 1.70, 2.00 and 2.30%) of phenylalanine and a fixed level (0.52%) of tyrosine (Table 1). The experimental diets contained casein and gelatin as natural protein sources to supply a minimum quantity of phenylalanine yet maximum quantities of several other amino acids. Crystalline L-amino acids were added to simulate the amino acid profile of *C. mrigala* muscle protein (Mohanty and Kaushik, 1991), except for the aromatic amino acids. The diets were made isonitrogenous by decreasing the non-essential amino acids as the level of phenylalanine increased. Dietary ingredients were mixed, adjusted to a pH of around 7.0 with NaOH, and pelleted (Benakappa and Varghese, 2002b)

Experimental design and feeding. Hatchery-raised *C. mrigala* juveniles were procured from Karnataka State Fish Seed Farm, Bhadra Reservoir Project, India. Prior to the start of the experiment, fish were acclimated to laboratory conditions for about 12 days, during which time they were fed a 40% protein diet. The experiment was conducted in 18 flow-through fiberglass tanks of 120 l with a water flow rate of approximately 0.5 l/min. Supplemental aeration was provided for each tank and incandescent lighting provided a diurnal light:dark cycle of 12:12 h.

At the start of the experiment, *C. mrigala* juveniles were sorted into groups of 20 fish and randomly stocked into the 18 tanks. The initial mean weight of the juveniles was 0.92 g. Triplicate groups were fed the respective experimental diets at a rate of 10% of the body weight per day, divided into two equal feedings (9:00, 15:00) for 56 days. The groups were weighed every week and the amount of feed was adjusted accordingly.

Prior to the morning feeding, tanks were cleaned by siphoning excess feed and fecal matter that had accumulated on the bottom. During the weekly samplings, the tanks were washed thoroughly and filled with fresh water.

Water quality. Water quality was analyzed during the weekly samplings. Temperature, pH, dissolved oxygen, total ammonia, free carbon dioxide and total alkalinity were measured (APHA, 1995). Water temperature ranged 27.5-28.6°C, alkalinity 45-71 ppm, dissolved oxygen 7.8-9.9 mg/l, carbon dioxide 0-0.3 mg/l, pH 6.8-7.7 and total ammonia 0.35-1.73 µg N/l. The water quality parameters were within favorable limits for carp growth (Jhingran, 1991).

Table 1. Composition (%) of test diets.

Common Ingredients	*Composition (% of total diet)*
Dextrin	25.00
Cod liver oil	5.00
Sunflower oil	5.00
Vitamin mix[1]	2.00
Mineral mix[1]	4.00
DLα-tocopherol acetate	0.01
BHA	0.02
Carboxymethyl cellulose	5.00
Cellulose	8.00
Casein	10.00
Gelatin	25.00
EAA mix[2]	6.97

Variable ingredients	*Diet (%)*					
	1	*2*	*3*	*4*	*5*	*6*
Non-EAA mix[3] (% of diet)	4.00	3.70	3.40	3.10	2.80	2.50
Phenylalanine supplement (% of diet)	0	0.30	0.60	0.90	1.20	1.50
Total phenylalanine (% of diet)	0.80	1.10	1.40	1.70	2.00	2.30
Total tyrosine (% of diet)	0.52	0.52	0.52	0.52	0.52	0.52
g phenylalanine/100 g protein	2.00	2.75	3.50	4.25	5.00	5.75
g phenylalanine + tyrosine/100 g protein	3.30	4.05	4.80	5.55	6.30	7.05
Crude protein (%) analyzed	40.18	40.22	40.31	40.29	40.34	40.17

[1] Benakappa and Varghese, 2002a.
[2] Essential amino acid mix (g/100 g dry diet 1): arginine 0.56, histidine 0.88, isoleucine 0.76, leucine 1.41, lysine 1.69, methionine 0.03, threonine 0.78, valine 0.67, tryptophan 0.19.
[3]Non-EEA mix (g/100 g dry diet): cystine 0.02, alanine 0.28, aspartic acid 1.87, glutamic acid 1.28, serine 0.55.

Chemical analysis. AOAC (1995) methods were used for proximate analysis of ingredients and diets. Amino acid contents of ingredients and diets were analyzed with an amino acid analyzer (LKB model 4050 Alpha Plus)

Statistical analysis. The mean weight gains at the end of the experiment were tested using two-way analysis of variance (Snedecor and Cochran, 1968). Duncan's multiple range test was employed to determine the statistical significance of differences among treatments. The optimum dietary requirements of *C. mrigala* for aromatic amino acids were determined by fitting the weight gain of the fish to breakpoint regression analysis (Robbins et al., 1979).

Results

The mean weight gains, feed conversion rates, specific growth rates and survival are presented in Table 2. The fish fed diet 1 grew poorly and suffered higher mortality than the fish fed the other five diets. Mean weight gains increased significantly as the phenylalanine level was raised to 1.70% of the diet. Beyond this level, growth decreased. The poor growth recorded for fish fed diets with less than 1.70% phenylalanine indicates that phenylalanine is indeed essential for *C. mrigala* growth. The highest specific growth rate and survival and lowest feed conversion ratio were obtained with diet 4. The breakpoint in the growth curve occurred at 1.75% dietary phenylalanine (Fig.1). Accordingly, this level (corresponding to 4.38% of the dietary protein) is considered the optimum dietary concentration for growth and survival of *C. mrigala* juveniles in the presence of 0.52% tyrosine. The optimum total aromatic amino acid (phenylalanine + tyrosine) was estimated as 2.27% of the diet, or 5.68% of the dietary protein.

Discussion

The total aromatic amino acid requirement of *C. mrigala* determined in the present study (5.68% of the dietary protein) is comparable to

Table 2. Weight gain, specific growth rate (SGR), food conversion rate (FCR) and survival of *C. mrigala* fed graded levels of phenylalanine*.

Phenylalanine level							
% of diet	*% of dietary protein*	*Mean initial weight (g)*	*Mean final weight (g)*	*Mean weight gain (%)*	*SGR (%)*	*FCR*	*Survival (%)*
0.80	2.00	0.92±0.01	1.27±0.01	38.04[a]	0.84	17.00	76.66
1.10	2.75	0.93±0.01	1.61±0.01	73.11[b]	0.98	9.73	83.33
1.40	3.50	0.92±0.01	2.06±0.02	123.91[c]	1.44	6.66	96.66
1.70	4.25	0.90±0.01	2.87±0.03	218.88[d]	2.03	4.95	100.00
2.00	5.00	0.92±0.01	2.61±0.03	183.6[e]	1.86	5.28	100.00
2.30	5.75	0.92±0.01	2.41±0.02	161.95[f]	1.72	5.78	93.33

Values with different superscripts differ significantly (p<0.05).
* Values are means of three replicates of 20 fish each.

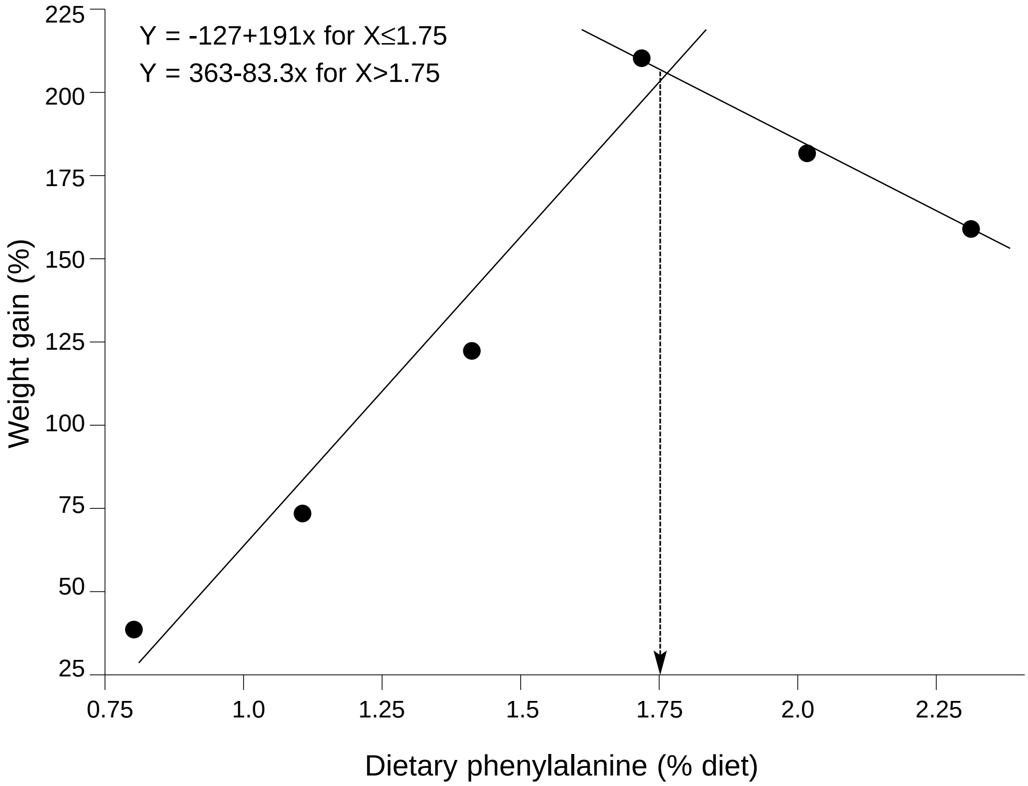

Fig.1. Optimum dietary phenylalanine level, determined by breakpoint analysis.

that of rohu (5.53%; Murthy and Varghese, 1996), *C. mrigala* (5.77%; Murthy, 2002), eel (5.6%; NRC, 1993) and Nile tilapia (5.5%; Santiago and Lovell, 1988), but lower than reported values for common carp (6.0%; Nose, 1979) and catla (6.2%; Ravi and Devaraj, 1991) and higher than values for channel catfish (5.0%; Robinson et al., 1980), rainbow trout (5.2%; Ogino, 1980), Mozambique tilapia (2.5%; Jauncey et al., 1983) and milkfish (5.2%; Borlongan and Coloso, 1993).

The wide variation in EAA requirements among species may be due to differences in methodologies used, such as the nature of the dietary protein source in the test diets, environmental conditions, experimental design, fish of different ages, sizes or strains (Borlongan, 1991; Moon and Gatlin, 1991; Cowey, 1994).

The replacement value of tyrosine for total aromatic amino acid was reported to be 42% for young chicks (Sasse and Baker, 1972) and 49% for weanling pigs (Robbins and Baker, 1977). Tyrosine has been found to replace 50% of total aromatic amino acids in channel catfish (Robinson et al., 1980), Nile tilapia (Santiago and Lovell, 1988), milkfish (Borlongan and Coloso, 1993) and rohu (Murthy and Varghese, 1996). In the present study, the sparing effect of tyrosine for phenylalanine was not investigated, however, it was presumed to be around 50%.

The growth of *C. mrigala* decreased when phenylalanine exceeded the required level. The reduction in growth may be attributed to amino acid catabolism. Probably, the excessive level of aromatic amino acids led to accumulation and oxidation of phenylalanine to ketones and other toxic metabolites, adversely affecting growth. Harper et al. (1970) and Austic (1978) reported that excessive levels of amino acids may become toxic and adversely affect growth because disproportionate intake affects the absorption and utilization of other amino acids. They also opined that a balance of required amino acids is needed for maximum utilization. Growth of *C. mrigala* dropped when the test amino acid exceeded its requirement. The decrease in growth is attributed to the use of energy in nitrogenous excretion, because excess amino acid is deaminated and excreted in the form of ammonia (Walton, 1985).

Except for a loss of appetite, which resulted in low food intake and depressed growth, no nutritional pathology was observed with the aromatic amino acid deficient diets.

Results of the present study clearly demonstrate the essentiality of aromatic amino acids for *C. mrigala* juveniles. Information on the estimated dietary requirement is useful in formulating diets balanced in phenylalanine and tyrosine for producing *C. mrigala*, particularly under controlled conditions.

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